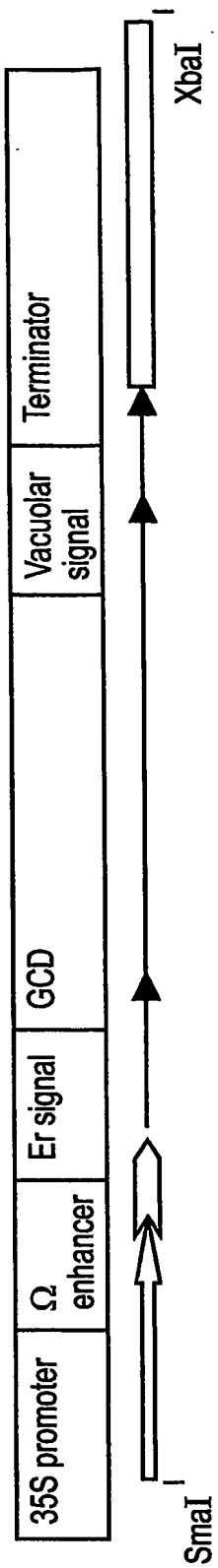

35S promoter
Ω enhancer
Er signal
GCD
Vacuolar signal
Terminator
SmaI
XbaI

Fig. 1a

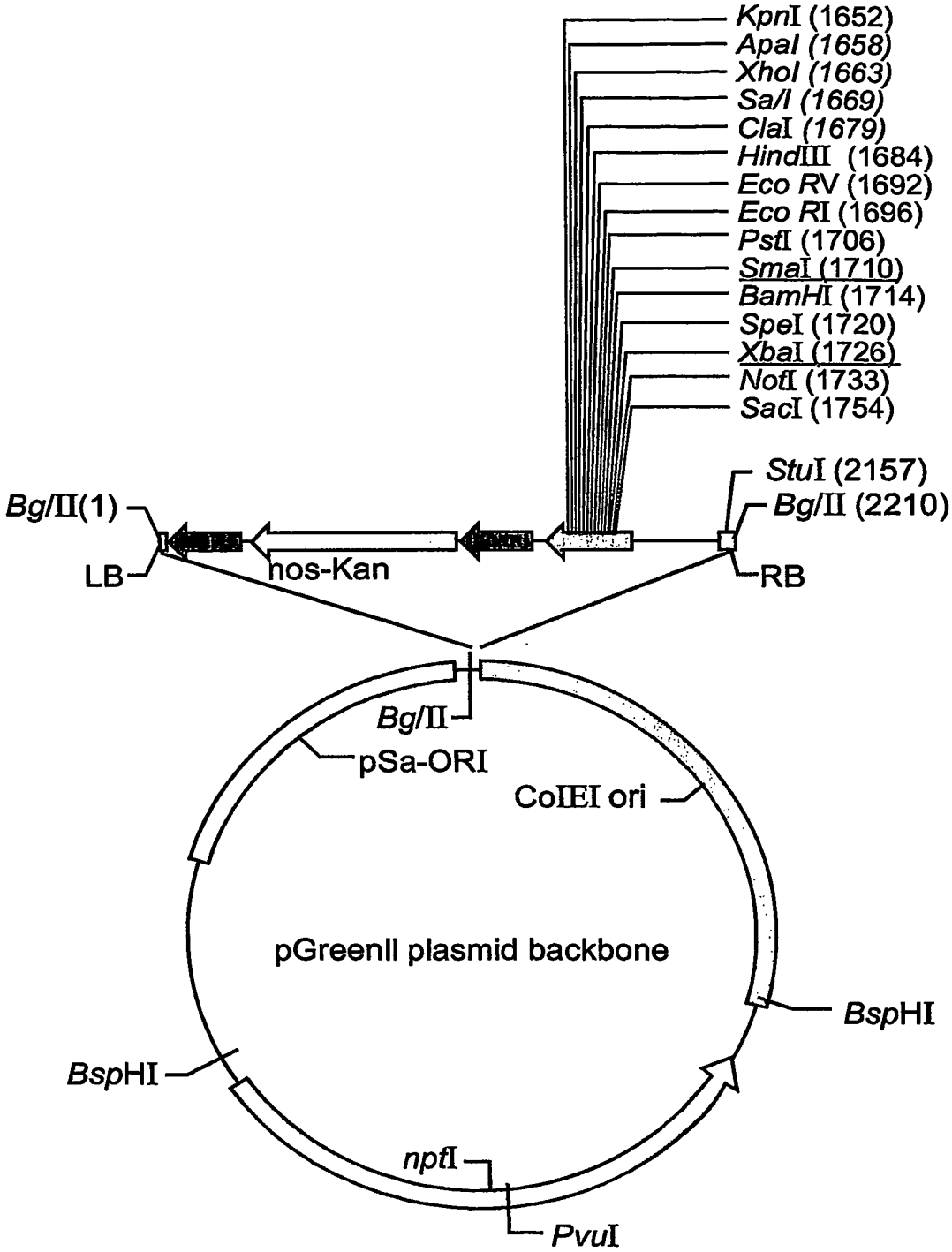

KpnI (1652)
ApaI (1658)
XhoI (1663)
SalI (1669)
ClaI (1679)
HindIII (1684)
Eco RV (1692)
Eco RI (1696)
PstI (1706)
SmaI (1710)
BamHI (1714)
SpeI (1720)
XbaI (1726)
NotI (1733)
SacI (1754)
StuI (2157)
Bg/II (2210)
Bg/II(1)
LB
nos-Kan
RB
Bg/II
pSa-ORI
CoIEI ori
pGreenII plasmid backbone
BspHI
BspHI
nptI
PvuI

Fig. 1b

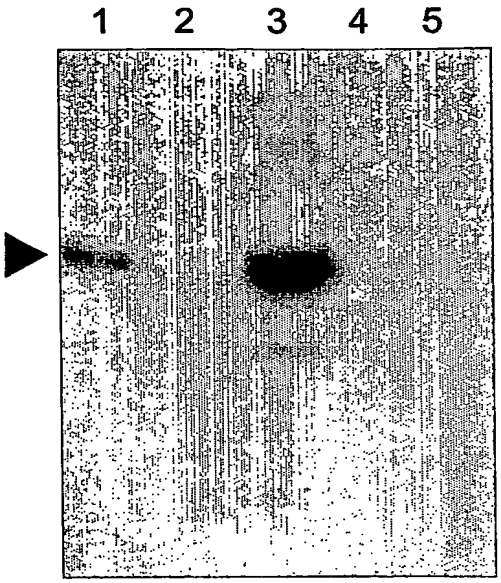


Fig. 2

Transformed cells express rGCD. 1 gram calli tissue was homogenized and 15 microgram of soluble cell extract were run on SDS-PAGE. Expression of rGCD in selected transformed calli was tested by western blot analysis with specific anti hGCD antibodies. 1: standard cerezyme, 2: untransformed callus extract, 3-5: various selected transformed calli extracts .

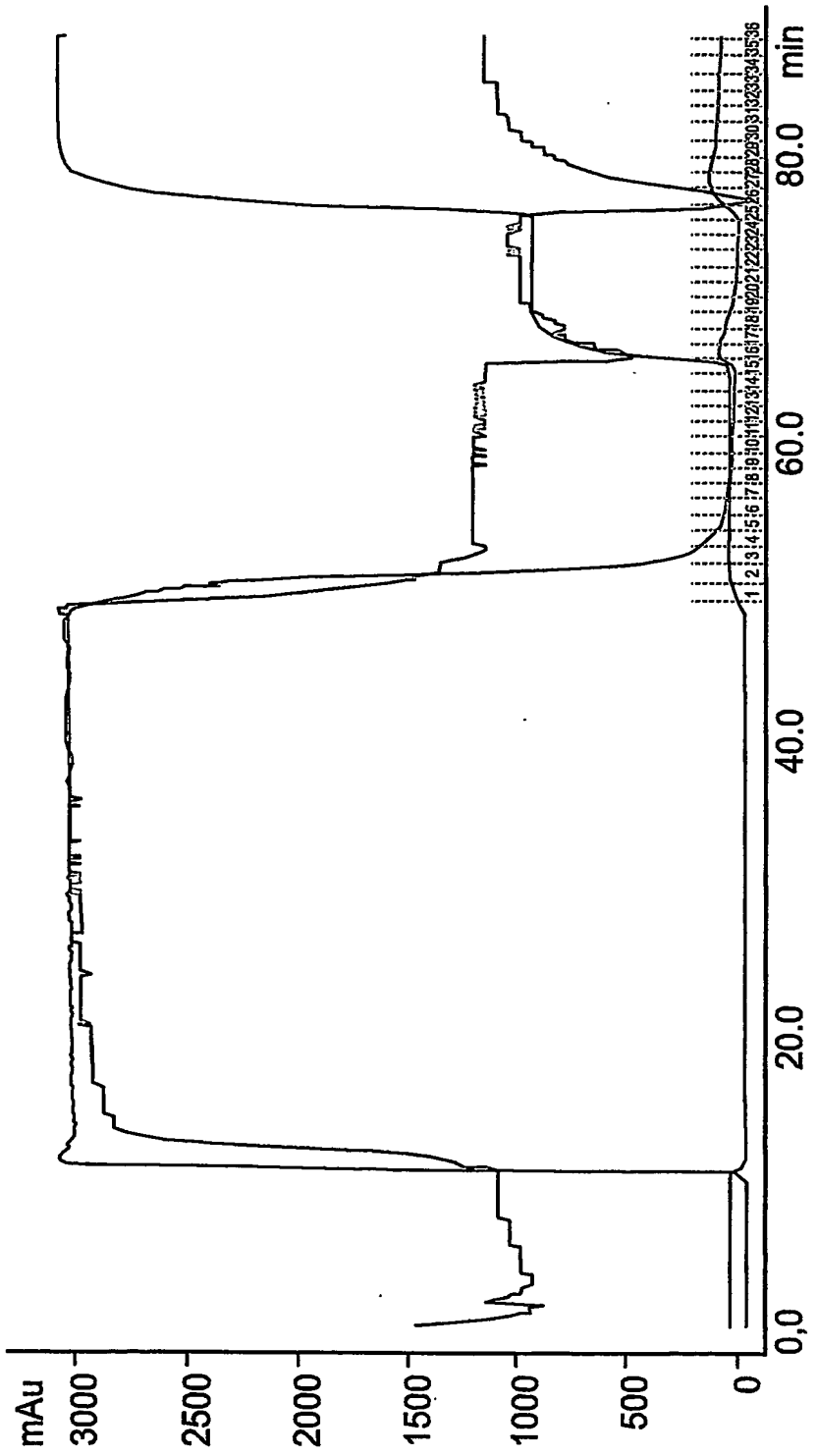

UV
Cond
pH
Fractions
mAu
3000
2500
2000
1500
1000
500
0
0,0
20.0
40.0
60.0
80.0
min

Fig. 3a

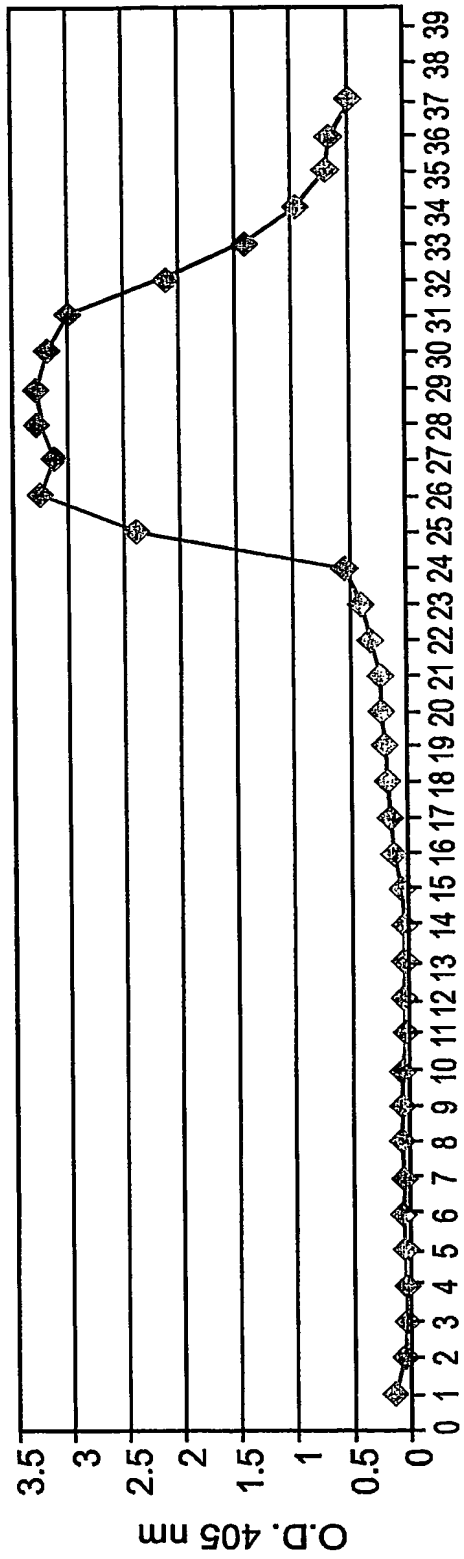

O.D. 405 nm
3.5
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Fraction

Fig. 3b

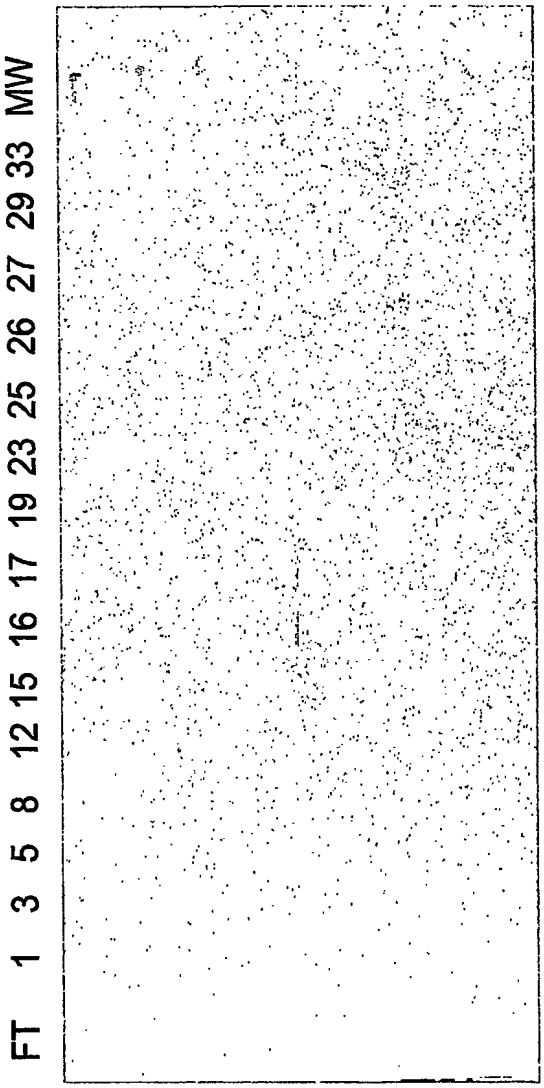

FT 1 3 5 8 12 15 16 17 19 23 25 26 27 29 33 MW

Fig. 3c

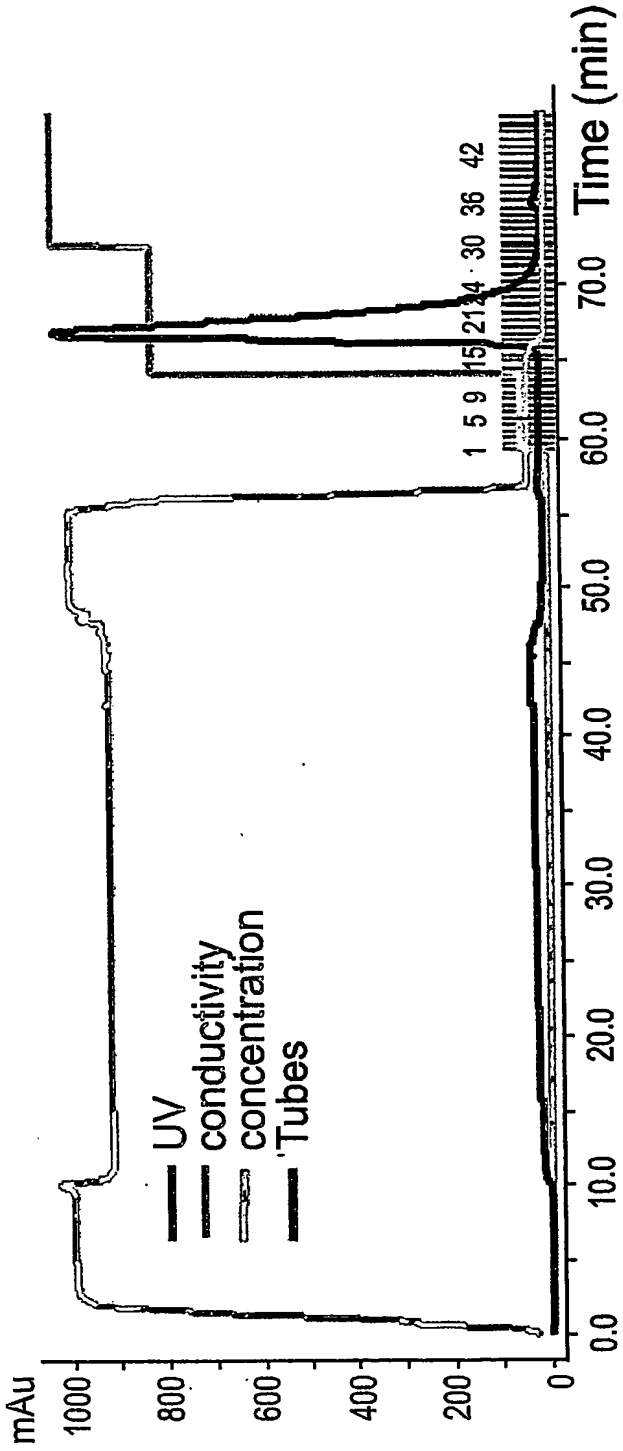

mAu
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Time (min)
1 5 9 15 21 24 30 36 42
UV
conductivity
concentration
Tubes

Fig. 3d

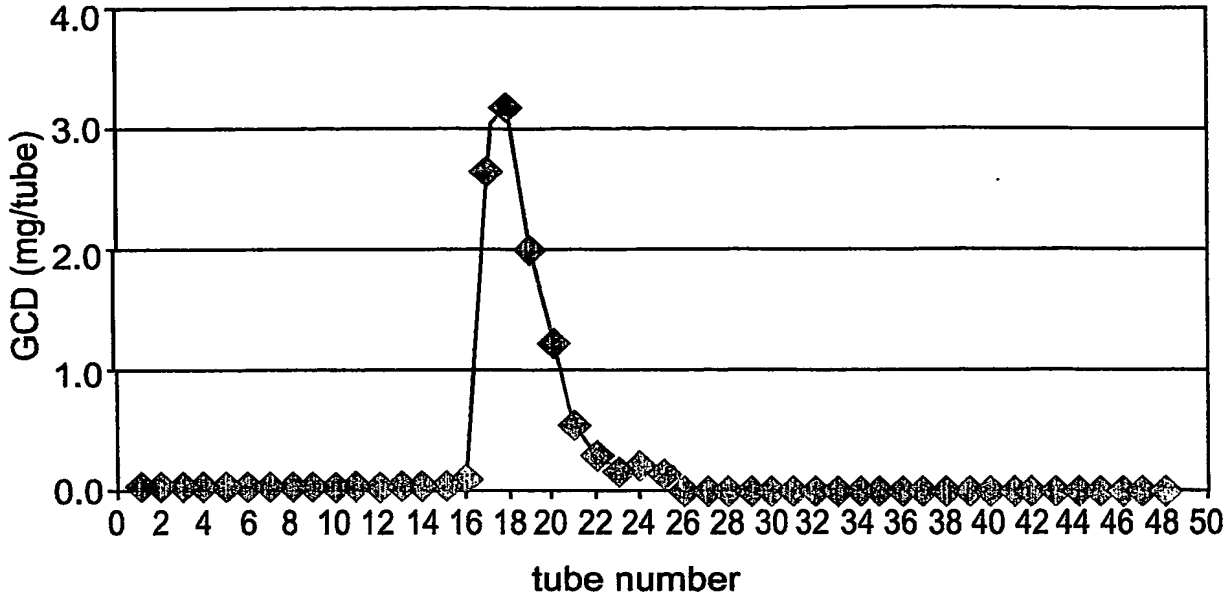

4.0
3.0
2.0
1.0
0.0
GCD (mg/tube)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
tube number

Fig. 3e

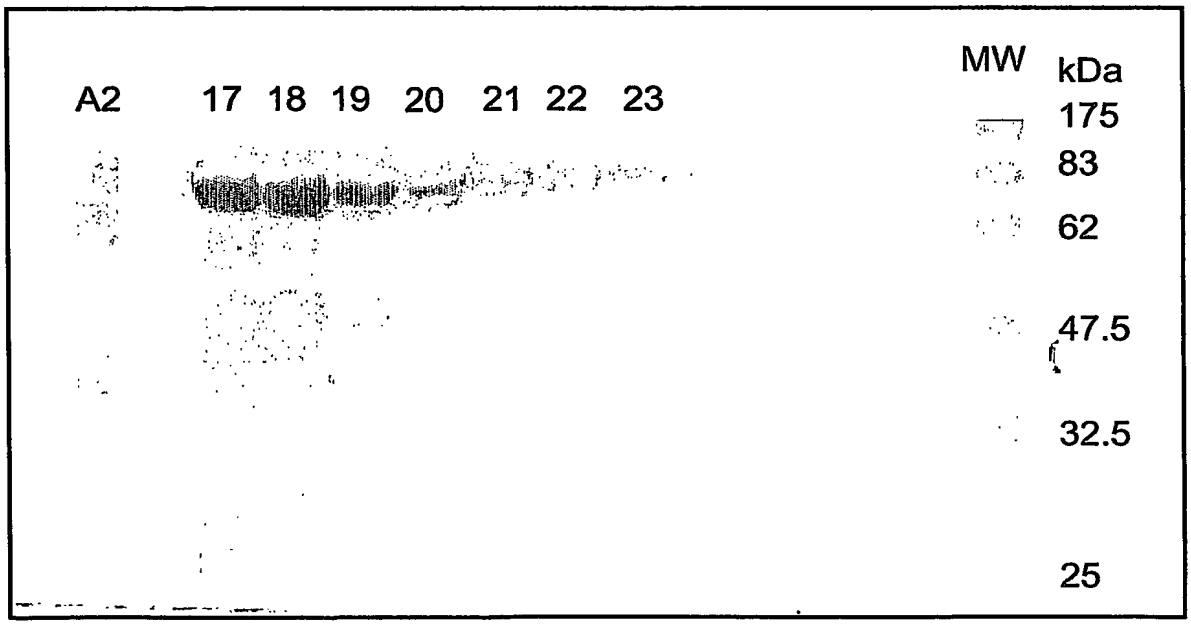

A2
17 18 19 20 21 22 23
MW kDa
175
83
62
47.5
32.5
25

Fig. 3f

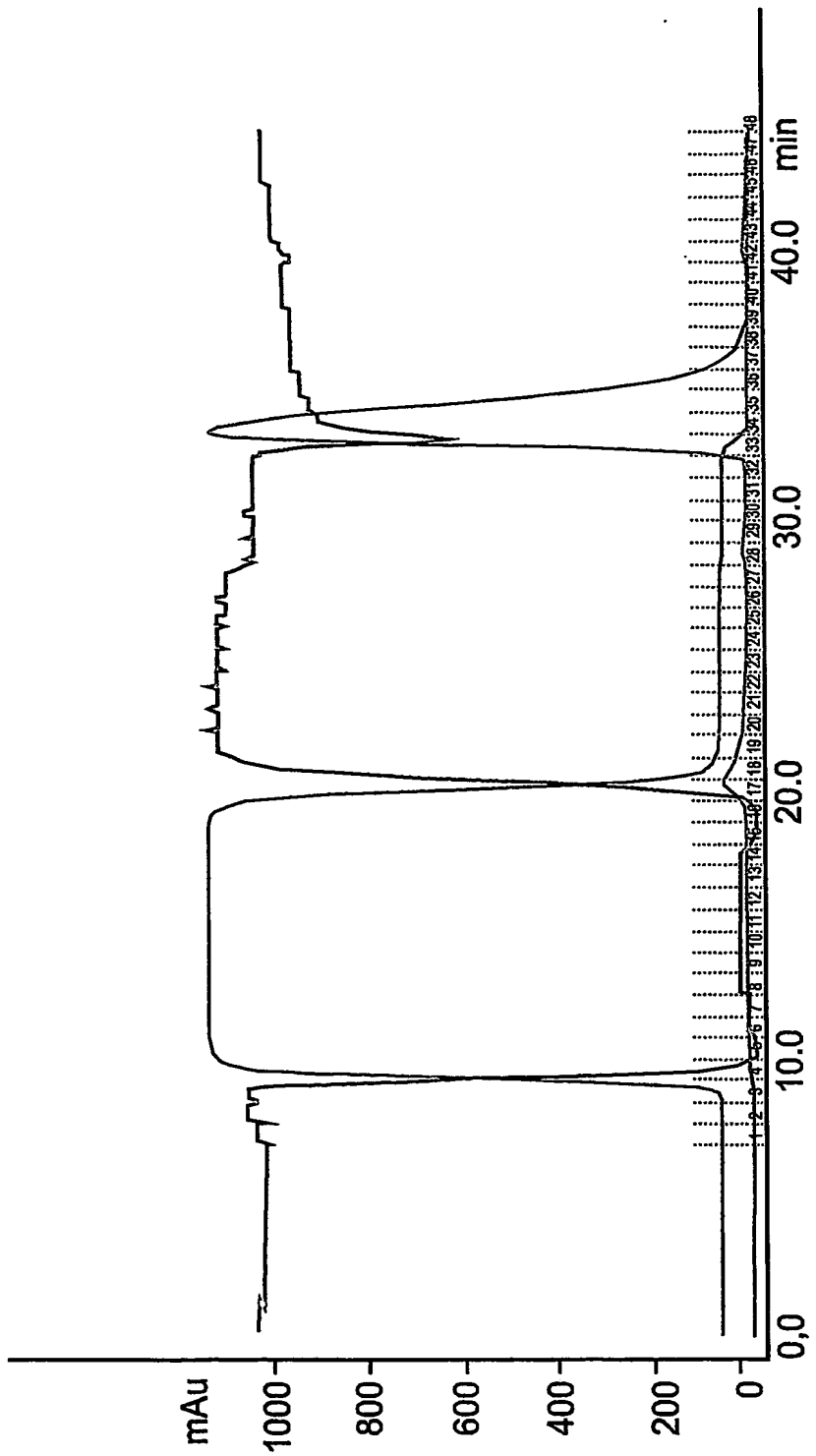

UV
Cond
pH
Fractions
mAu
1000
800
600
400
200
0
0,0
10.0
20.0
30.0
40.0
min

Fig. 4a

Fig. 4b

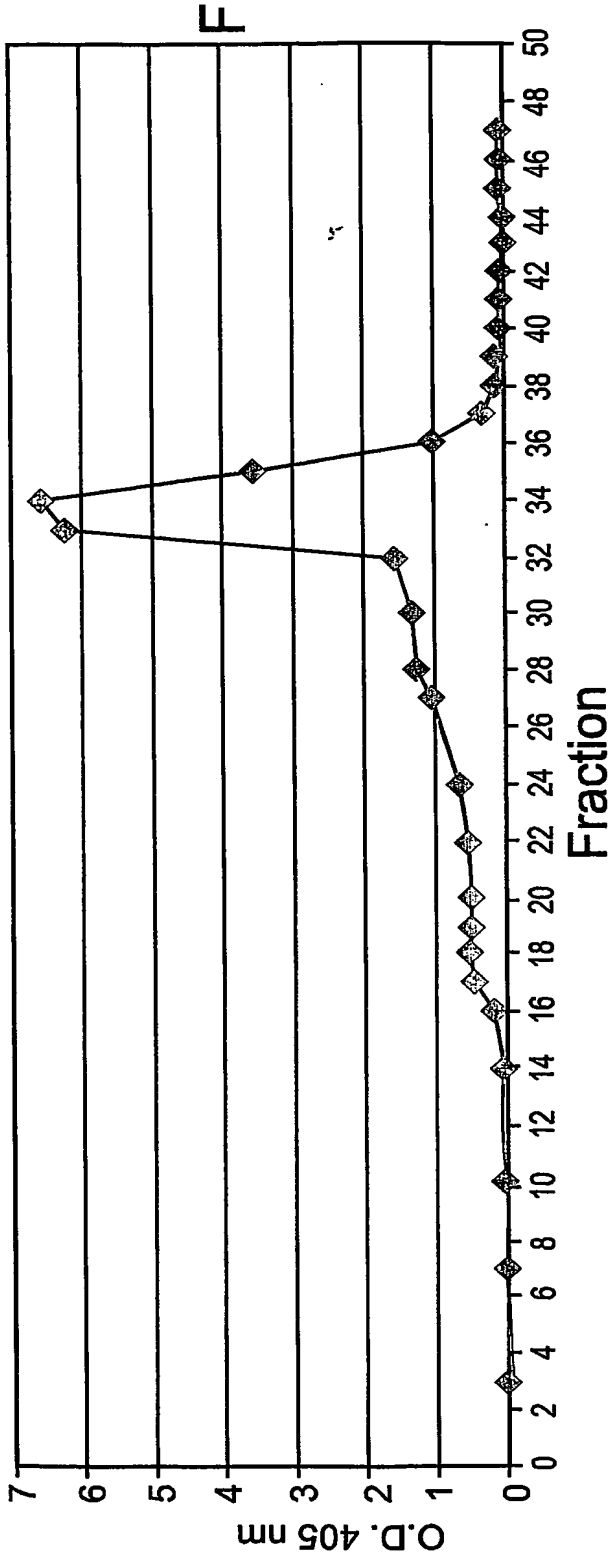
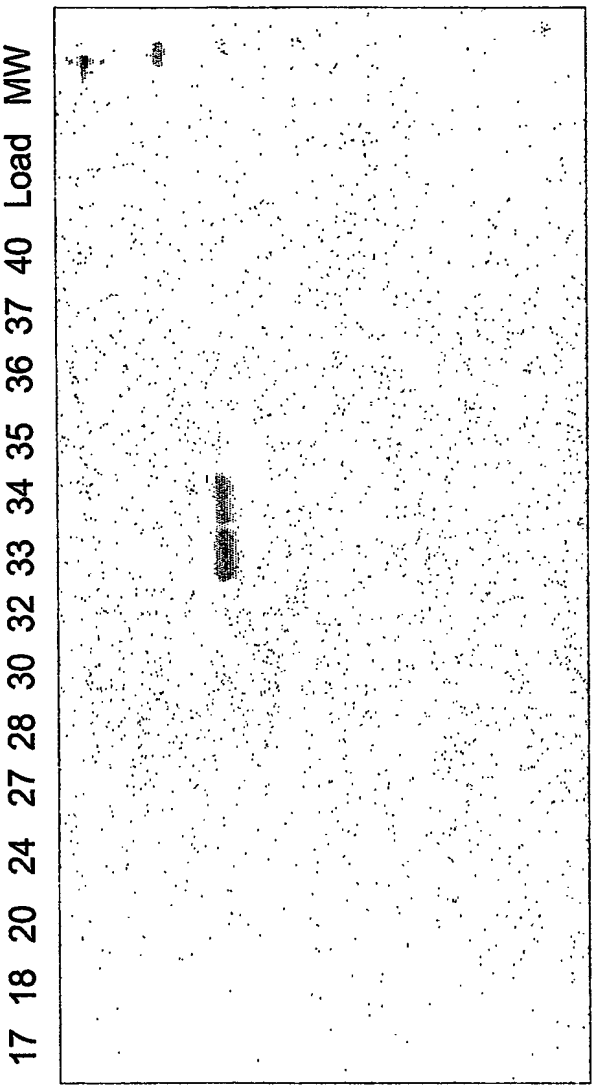

O.D. 405 nm
0 1 2 3 4 5 6 7
Fraction
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50

Fig. 4c


Load MW
17 18 20 24 27 28 30 32 33 34 35 36 37 40

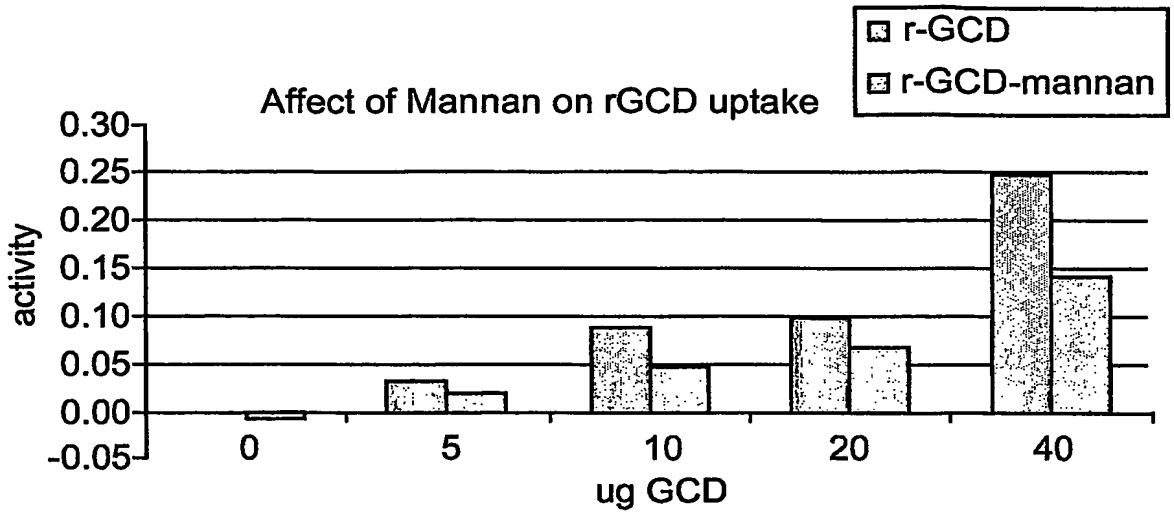

r-GCD
r-GCD-mannan
Affect of Mannan on rGCD uptake
activity
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
5
10
20
40
ug GCD

Fig. 5a

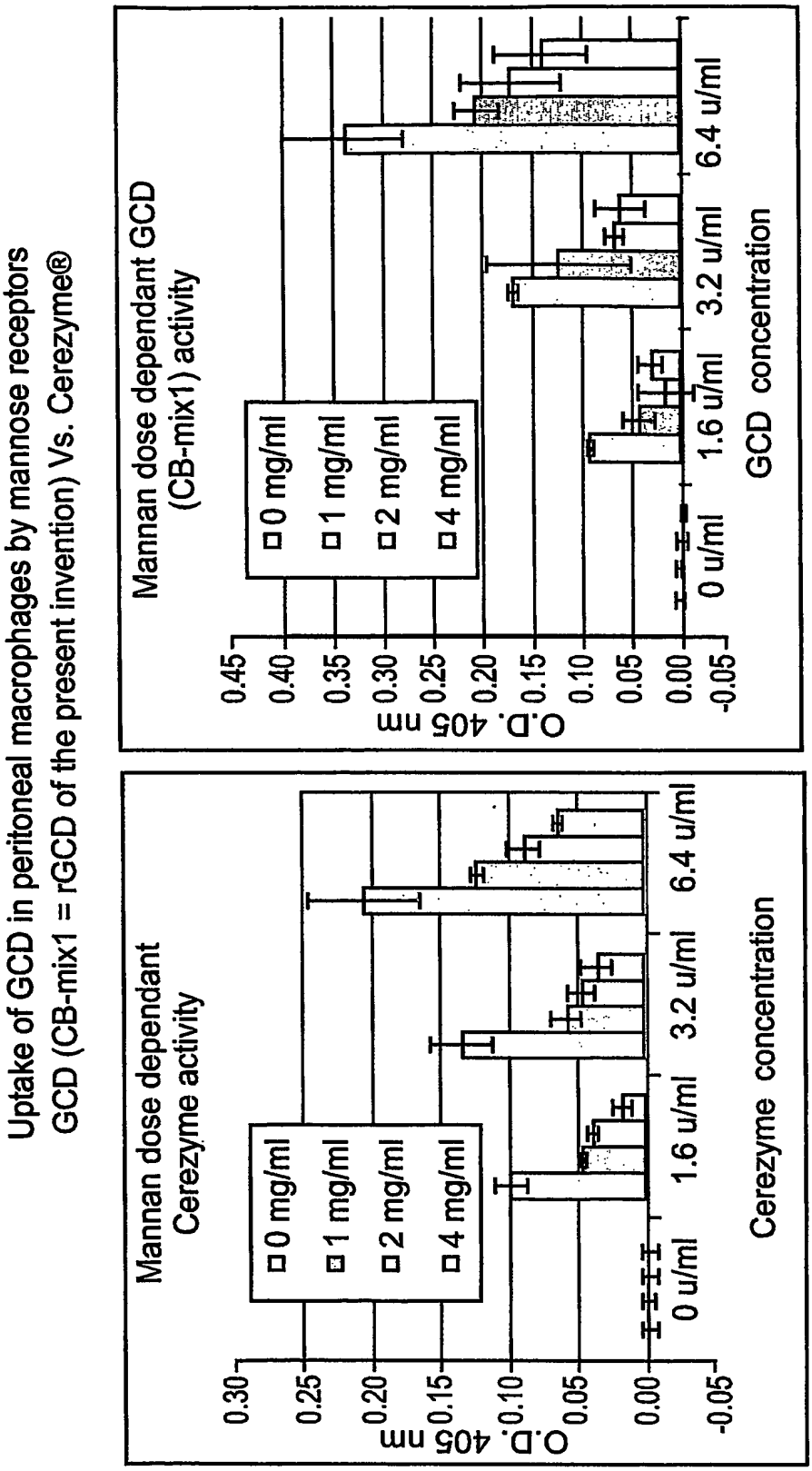

Uptake of GCD in peritoneal macrophages by mannose receptors
GCD (CB-mix1 = rGCD of the present invention) Vs. Cerezyme®
Mannan dose dependant GCD
(CB-mix1) activity
O.D. 405 nm
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0 mg/ml
1 mg/ml
2 mg/ml
4 mg/ml
0 u/ml
1.6 u/ml
3.2 u/ml
6.4 u/ml
GCD concentration
Mannan dose dependant
Cerezyme activity
O.D. 405 nm
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0 mg/ml
1 mg/ml
2 mg/ml
4 mg/ml
0 u/ml
1.6 u/ml
3.2 u/ml
6.4 u/ml
Cerezyme concentration

Fig. 5b

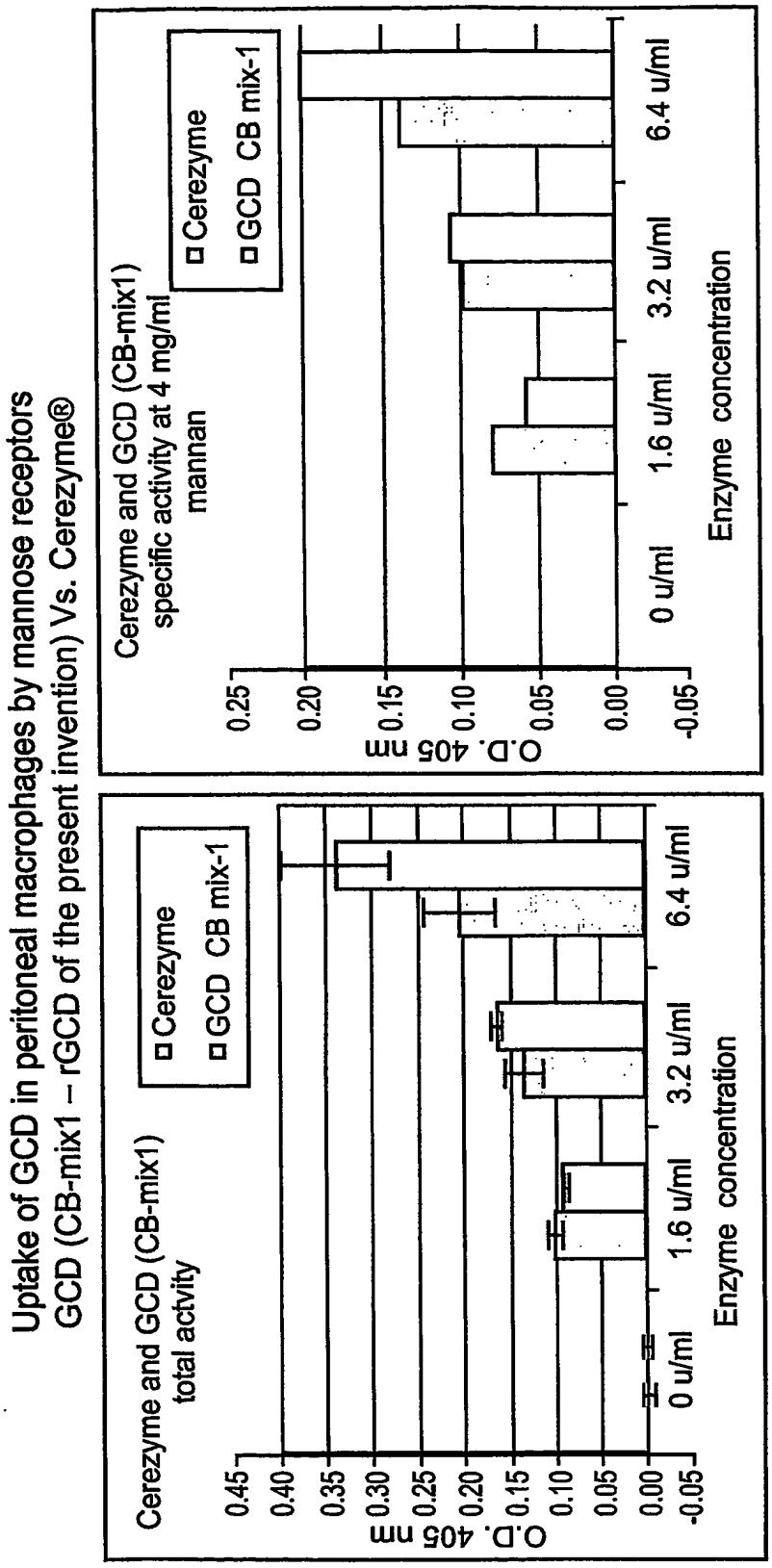

Uptake of GCD in peritoneal macrophages by mannose receptors
GCD (CB-mix1 – rGCD of the present invention) Vs. Cerezyme®
Cerezyme and GCD (CB-mix1)
total activity
Cerezyme
GCD CB mix-1
O.D. 405 nm
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0 u/ml
1.6 u/ml
3.2 u/ml
6.4 u/ml
Enzyme concentration
Cerezyme and GCD (CB-mix1)
specific activity at 4 mg/ml
mannan
Cerezyme
GCD CB mix-1
O.D. 405 nm
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0 u/ml
1.6 u/ml
3.2 u/ml
6.4 u/ml
Enzyme concentration

Fig. 5c

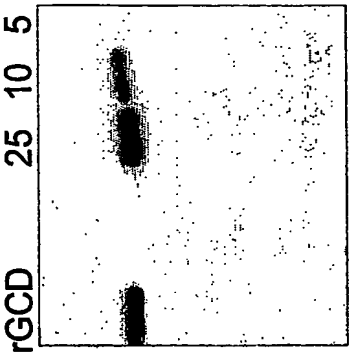
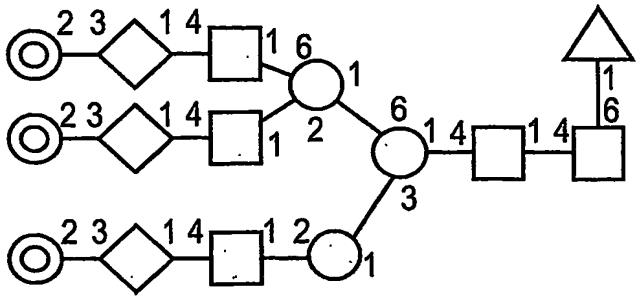
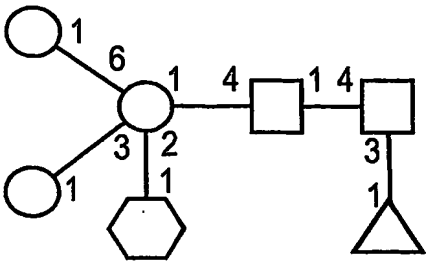

GCD standard (ng)
rGCD
25
10
5

Fig. 5d



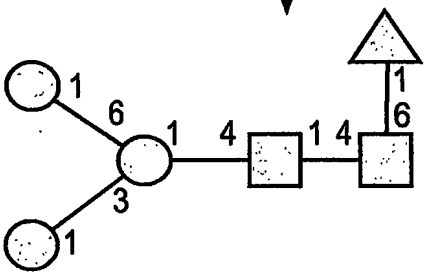
Major glycan structure from CHO cells



Major glycan structure from carrot cells:

Mannose terminal glycan

Glycan remodeling with glycosidases



Major remodeled glycan structure on Cerezyme

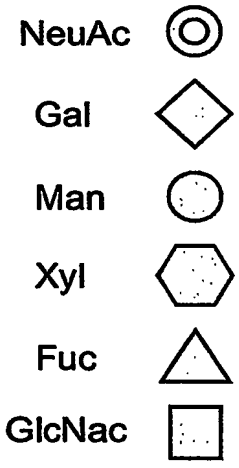


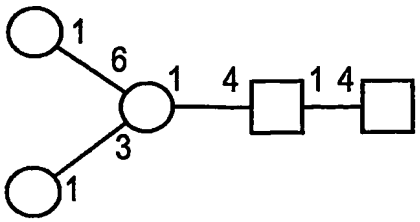
Fig. 6

a) Ma3---Mb4GNb4---GN
Ma6-|
Xb2-+ Fa3-+

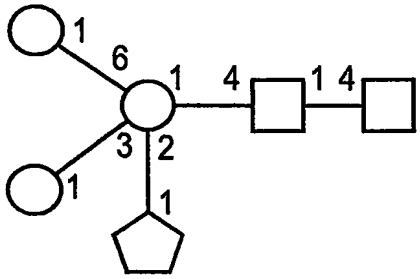
b) Ma3---Mb4GNb4GN
Ma6 -+

c) Ma3---Mb4GNb4---GN
Ma6-+
Fa3-+

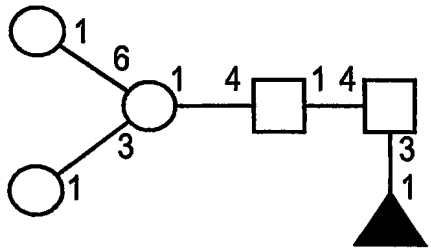
Fig. 7



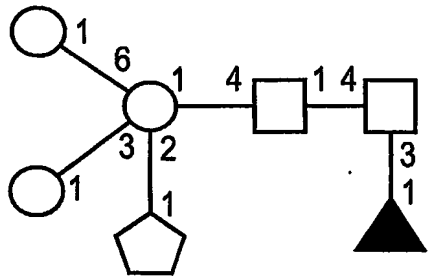
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1171.5



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1331.6

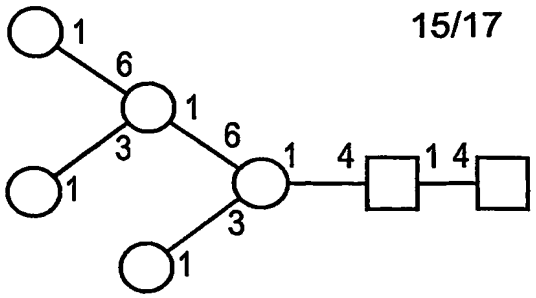


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1345.6

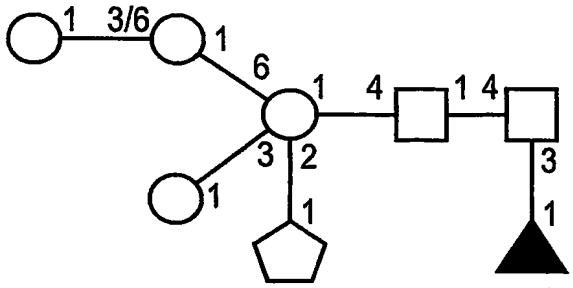


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1505.7

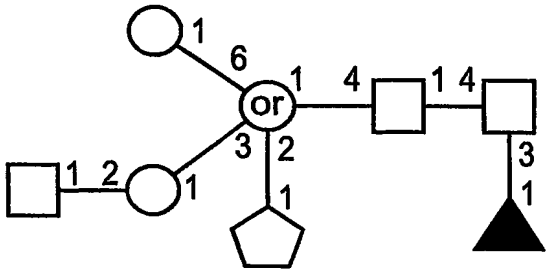
Fig. 8a



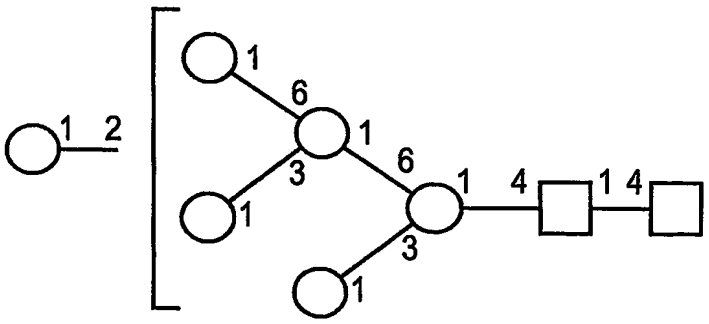
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1579.8



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1709.7

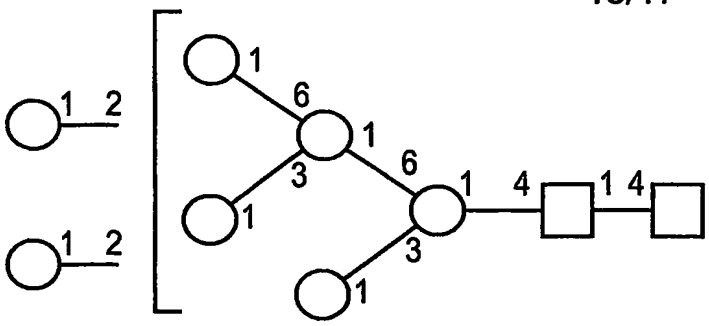


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1750.9

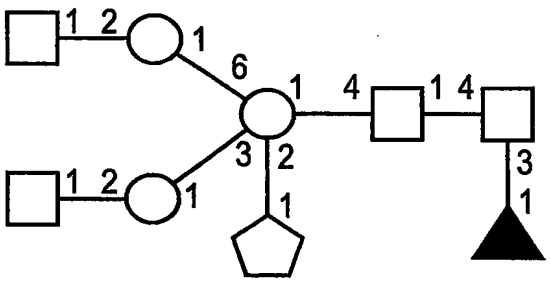


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1783.9

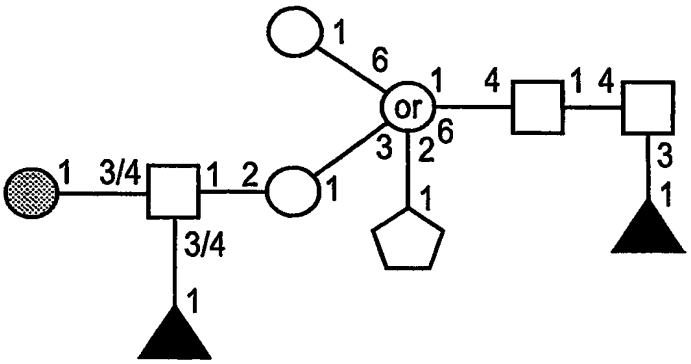
Fig. 8b



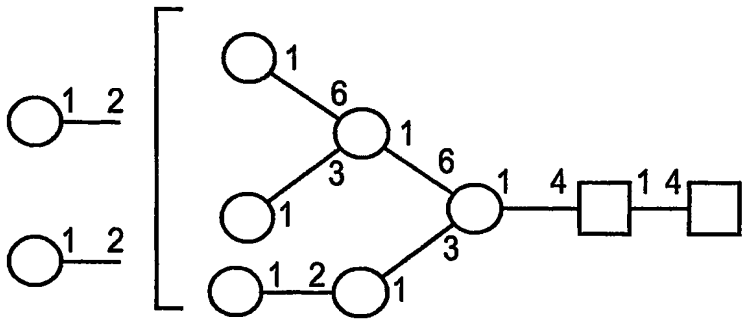
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1989.0



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1997.0

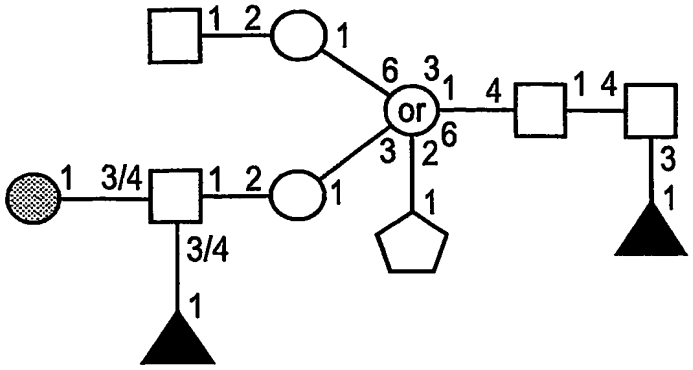


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2130.0

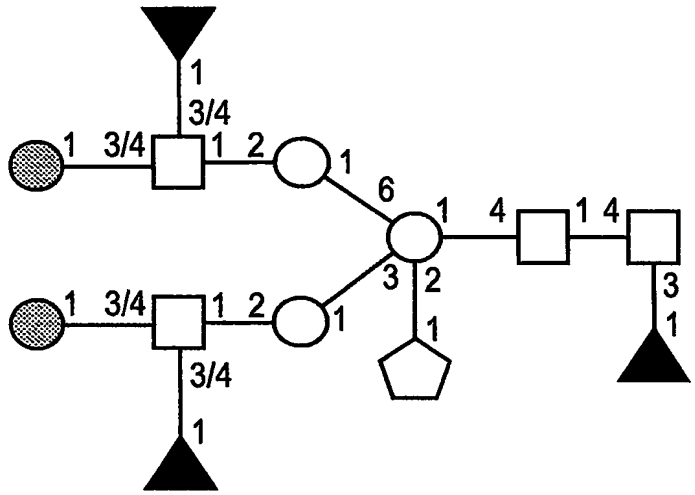


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2193.1

Fig. 8c



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2375.2



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2375.2

Key:

- Fucose
- Galactose
- N-Acetylglucosamine
- Mannose
- Xylose

Fig. 8d